

Report on Current and Future Biological and Ecosystem Science Research Activities

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PURPOSE

To provide the Scientific Review Board with a description of progress towards research activities described in the IPHC's five-year Program of Integrated Research and Monitoring (2022-2026).

BACKGROUND

The primary biological and ecological research activities at the IPHC that follow Commission objectives are identified and described in the <u>IPHC Five-Year Program of Integrated Research</u> and <u>Monitoring (2022-2026)</u>. These activities are integrated with stock assessment (SA) and the management strategy evaluation (MSE) processes (<u>Appendix I</u>) and are summarized in five main areas, as follows:

- <u>Migration and Population Dynamics</u>. Studies are aimed at improving current knowledge of Pacific halibut migration and population dynamics throughout all life stages in order to achieve a complete understanding of stock structure and distribution across the entire distribution range of Pacific halibut in the North Pacific Ocean and the biotic and abiotic factors that influence it.
- 2) <u>Reproduction</u>. Studies are aimed at providing information on the sex ratio of the commercial catch and to improve current estimates of maturity and fecundity.
- 3) <u>Growth</u>. Studies are aimed at describing the role of factors responsible for the observed changes in size-at-age and at evaluating growth and physiological condition in Pacific halibut.
- 4) <u>Mortality and Survival Assessment</u>. Studies are aimed at providing updated estimates of discard mortality rates in the guided recreational fisheries and at evaluating methods for reducing mortality of Pacific halibut.
- 5) <u>Fishing Technology</u>. Studies are aimed at developing methods that involve modifications of fishing gear with the purpose of reducing Pacific halibut mortality due to depredation and bycatch.

A ranked list of biological uncertainties and parameters for SA (<u>Appendix II</u>) and the MSE process (<u>Appendix III</u>) and their links to research activities and outcomes derived from the five-year research plan are provided.

SRB RECOMMENDATIONS AND REQUESTS

The SRB issued several recommendations and requests in their report of SRB023 (<u>IPHC-2023-SRB023-R</u>) in relation to presentation <u>IPHC-2023-SRB023-08</u>:

SRB023–Rec.12 (<u>para. 36</u>) **NOTING** that the genomics research is and will continue to be a key element of the Biological and Ecosystem Science Research program, and that the Secretariat wishes to (i) document stock structure, (ii) use genetic markers to quantify movements, (iii) assign individuals of any age, location, season to a genetic population, (iv) annotate markers and use genomic data to between understand genetic and environmental sources of variation in growth, maturity and fecundity, (v) engage in close-kin capturerecapture to estimate stock abundance, the SRB **RECOMMENDED** adding qualified staff to help address these diverse and important activities in a timely fashion.

The IPHC Secretariat is currently studying this recommendation in the context of the goals and objectives of the 5Y-PRIM 2022-2026.

SRB023–Rec.13 (para. 42) The SRB **RECOMMENDED** that the Secretariat continue to work with collaborators to collect and process genetic samples from juveniles. Collections of younger (pre-reproductive) age classes would be particularly important for anticipated future close-kin capture-recapture work.

The IPHC Secretariat has over the recent years collected genetic samples (fin clips) from juvenile Pacific halibut captured in the NMFS Bottom Trawl Survey in the Gulf of Alaska, Bering Sea and Aleutian Islands. This is the only source of juvenile Pacific halibut biological samples since the FISS captures typically fish that are 5-6 years of age and above. Unfortunately, the Commission did not fund the deployment of IPHC Staff in the NMFS Bottom Trawl Survey in 2024 and no Pacific halibut juvenile samples will be collected.

SRB023–Rec.14 (<u>para. 44</u>) The SRB **RECOMMENDED** to apply the genetic sampling more broadly, to estimate genetic diversity of the (sub)populations, for example through the effective number of breeding adults by cohort.

The Secretariat is not aware of software that is currently available for estimating these parameters directly from genotype likelihood data. That being the case, effort would need to be redirected to adapting existing methods that make use of called genotype data.

SRB023–Rec.15 (<u>para. 45</u>) The SRB **RECOMMENDED** that the compensatory assumption of the stock recruitment models be critically evaluated via a MSE stress test scenario in which recruitment is depensatory at some low spawning biomass.

The IPHC Secretariat is currently addressing this recommendation and results will be presented as part of the MSE presentation during SRB024.

SRB023–Rec.16 (<u>para. 49</u>) The SRB **RECOMMENDED** that Secretariat proceed to the next step of individual assignment based on K of 4 or K of 5. Based on the large number of loci with low levels of divergence among reporting regions (Manhattan plot in Figure 4 of paper <u>IPHC-2023-SRB023-08</u>) that posterior probabilities of cluster assignment (in a Bayesian context) may be low when all loci are used. The Secretariat should conduct a comparable analysis using only 'outlier loci'.

As part of the procedure for estimating admixture proportions, probabilistic cluster assignments were obtained for these K values. However, most individuals were classified

as unassigned (<u>Fig. 6</u> in this report). Additionally, we anticipate that false signals of structure or noise may be captured if this analysis was repeated with only 'outlier loci', similar to the results observed when a set of loci were selected to perform assignment testing (<u>Fig. 7</u> in this report).

SRB023–Rec.17 (para. 50) **RECOGNIZING** that future applications of 'outlier loci' to address SA and MSE objectives will necessitate development of more 'rapid screening approaches' and screening based on fewer loci, the SRB **RECOMMENDED** that the Secretariat work to identify the numbers of loci and locus characteristics (e.g. high levels of diversity and high level of allele frequency variation) so loci may be applied.

The IPHC Secretariat is investigating whether some additional optimization of the assignment testing could be done to determine if assignment accuracy increases with alternative SNP selection strategies.

SRB023–Rec.18 (para. 53) The SRB **RECOMMENDED** that the Secretariat:

- a) conduct simulations as a means of assessing the accuracy of group or admixture assignments;
- b) establish criteria for acceptable group assignment accuracy and that is relevant for assignment of individuals as a 'pure' or 'admixed'. Thus, observations, though made with some error would be used as 'observed' estimates to tally over space and across age classes.
- c) should evaluate what the uncertainty in classification (errors) will mean to their estimates. The SRB draws the Secretariat's attention to a widely cited paper by Manel et al. (2005) in Trends in Ecology and Evolution, where authors compare individual assignment tests to a widely used alternative method (mixed stock analysis). These authors point out that use of individual assignment tests for relative population (or reporting group) compositional estimation can be fraught with problems because assignment error compounds across all individuals.

The IPHC Secretariat has addressed the potential application of cross-validation techniques such as leave-one-out as previously mentioned in SRB022-Rec.20 b) (para. 47) in reference to the proposed assignment testing. Leave-one out has been shown to upwardly bias accuracy assessments of these methods especially when loci are selected on the basis of allele frequencies (Anderson 2010). Therefore, we chose a more conservative approach and followed a training and holdout procedure for assessing assignment testing accuracy (Fig. 7 in this report). For the purposes of admixture assignments (unsupervised clustering) we required at least a membership probability of at least 0.8 and for group assignments (assignment testing) we required at least 0.95. The Secretariat thanks the SRB for the literature recommendation and insight.

SRB023–Req.01 (para. 37) **NOTING** that future applications of genomic data will necessitate more expansive sampling geographically and demographically to achieve IPHC goals, the SRB **REQUESTED** that the Secretariat establish explicit long-term objectives for use of genomic data and work with staff, fishermen, and agency collaborators to establish a short and long-term sampling program and data and sample archival plan to ensure samples are available to address Secretariat objectives.

The IPHC Secretariat is currently implementing long-term objectives for the collection of genetic samples coastwide that include the collection of fin clips from sampled commercial landings (since 2017; used to generate sex ratio information by genotyping), from all fish sampled in the FISS (since 2016) and from all research projects that have involved the capture of Pacific halibut (since 2016). An important source of genetic samples from juvenile Pacific halibut derives from the NMFS Ground Trawl Survey in the Gulf of Alaska, Bering Sea and Aleutian Islands (since 2019). Unfortunately, the Commission did not fund the deployment of IPHC Staff in the NMFS Bottom Trawl Survey in 2024 and no juvenile Pacific halibut samples will be collected this year.

SRB023–Req.02 (para. 41) **NOTING** paper <u>IPHC-2023-SRB023-08</u> (subsection 1.1 - Identification of Pacific halibut juvenile habitat), and that the narrative describes work to be conducted but does not explicitly identify research objectives or hypotheses that the data would be used to address, the SRB **REQUESTED** that objectives/hypotheses be developed for SRB024 where hypotheses could include: a) regions with larger amounts of juvenile rearing habitat and larger number of juveniles would realize numerically larger levels of recruitment to the adult population; b) genotypes of juveniles from rearing habitats could be assigned to specific spawning areas.

The IPHC Secretariat conducted initial work on Pacific halibut juvenile habitat identification with the involvement of the 2023 IPHC Intern and is in the process of investigating avenues to continue this work.

SRB023–Req.03 (<u>para. 43</u>) **NOTING** paper <u>IPHC-2023-SRB023-08</u> (subsection 1.2 - wire tagging of U32 Pacific halibut), where the narrative describes numbers of fish tagged and recovered, no information is provided summarizing distances moved by size/age and location, the SRB **REQUESTED** that information be provided during SRB024, including background on statistical methods for analysis of data.

The IPHC Secretariat will provide information on movement of tagged fish and plans to use these data to inform on survival during SRB024.

SRB023–Req.04 (<u>para. 51</u>) The SRB **ACKNOWLEDGED** Table 1 in paper <u>IPHC-2023-SRB023-</u> <u>08</u>, produced in response to SRB022 inquiry, and that discrepancies in the genetic diversity measure Fis (deviation of observed and expected heterozygosity) across collection years within reporting regions. The Secretariat estimates Fis on a collection year by year basis and overall years for each region. The SRB **REQUESTED**: a) further investigation of the disparity in Fis for reporting regions (yearly vs total). Higher positive Fis could indicate admixture of individuals from genetically differentiated groups; b) investigations into discrepancies between estimates of Fis, observed heterozygosity (Ho), and expected heterozygosity (He).

The disparity in yearly versus total F_{IS} for reporting regions is likely an artifact related to the fact that these values are summarized for all of the SNPs discovered using all of the individuals in dataset as a whole (n=570). We would like to note that we required that SNPs have a minor allele frequency (MAF) of at least 0.01, corresponding to an allele being observed at least 5 times when the sample size is 570. At the individual sample collection level, it is possible for a SNP that is detected in the entire dataset to go unobserved if the 5 occurrences of that allele are observed in other sample collections. Filtering SNPs at the sample collection level by requiring alleles to be observed at least 3 times appears to fix this issue to some extent (Table 1 in this report), but by doing so it means that genetic diversity is summarized for a subset of SNPs specific to each sample collections.

We would like to thank the SRB for pointing out the discrepancies related to b), as in the version of the table presented at SRB023 the columns Ho & He were mislabeled. Table 1 in this report has been revised to correct this error. In the previous table, arithmetic means were used to summarize of these metrics across all SNPs. Noting that the calculation of F_{IS} $(F_{IS} = 1 - (H_o/H_e))$ contains a ratio and therefore $\overline{F_{IS}} \neq 1 - (\overline{H_o}/\overline{H_e})$. A weighted mean (by H_e) has been included in Table 1 as well.

SRB023–Req.05 (para. 52) The SRB **NOTED** that the Secretariat proposes to conduct individual admixture (i.e. among IPHC reporting regions) estimation using software NGSadmix and individual assignment testing using WGSassign, both of which are amenable to low coverage sequence data, to estimate proportional contributions of reporting groups to unknown individuals. This analysis would be conducted after 'best supported' number of genetic groups (K) has been established. The SRB **REQUESTED** that admixture analyses and assignment testing be conducted and reported at SRB024, including estimates of assignment accuracy.

The unsupervised clustering methods we have presented to date, including that implemented in NGSadmix, have failed to identify discrete genetic clusters and at this point, the best supported value for K is 1. Furthermore, the interrogation of individual assignment probabilities to the genetic groups associated with the various K values testing using NGSadmix, led to most individuals being classified as un-assigned or admixed, in all cases (Fig. 6 in this report). Therefore, we feel it is not appropriate to proceed with establishing reporting groups based on these unsupervised clustering methods when a clear determination on the true number of clusters cannot be made or is not well supported by established model selection metrics or other criteria (Figs. 3 and 5 in this report). We did attempt to establish reporting groups based on the sampling localities and proceeded with conducting assignment testing. We used a simple, training and holdout cross-validation procedure to estimate a 34% assignment accuracy.

UPDATE ON PROGRESS ON THE MAIN RESEARCH ACTIVITIES

1. <u>Migration and Population Dynamics</u>.

The IPHC Secretariat is currently focusing on studies that incorporate genomics approaches in order to produce useful information on population structure, distribution and connectivity of Pacific halibut. The relevance of research outcomes from these activities for stock assessment (SA) resides (1) in the introduction of possible changes in the structure of future stock assessments, as separate assessments may be constructed if functionally isolated components of the population are found (e.g. IPHC Regulatory Area 4B), and (2) in the improvement of productivity estimates, as this information may be used to define management targets for minimum spawning biomass by Biological Region. These research outcomes provide the second and third top ranked biological inputs into SA (Appendix II). Furthermore, the relevance of these research outcomes for the MSE process is in biological parameterization and validation of movement estimates, on one hand, and of recruitment distribution, on the other hand (Appendix III).

1.1. <u>Population genomics</u>. The primary objective of these studies is to investigate the genetic structure of the Pacific halibut population and to conduct genetic analyses to inform on Pacific halibut movement and distribution within the Convention Area

Details on sample collection, sequencing, bioinformatic processing and proposed analyses utilizing low-coverage whole genome sequencing (IcWGR) to investigate Pacific halibut population structure were provided in documents <u>IPHC-2021-SRB018-08</u>, <u>IPHC-2022-SRB021-09</u> and <u>IPHC-2023-SRB022-09</u>.

1.1.1. Methods

Additional SNP filtering prior to summarizing genetic diversity metrics for each sample collection has been conducted to address disparities in Table 1 of document IPHC-2023-SRB023-08. In the previously reported table, arithmetic means were used to summarize of these metrics across all SNPs. discovered using all individuals in the dataset (n=570). We have now revised this table to summarize these values only for SNPs that are variable in each sample collection by requiring a minor allele count (MAC) of three within a collection for a SNP to be included (Table 1). Count based filtering may be better suited to smaller sample sizes (or when samples are partitioned into smaller groups). For a minor allele count of three, the rare allele will be observed in at least two individuals regardless of sample size. Summarizing diversity metrics when filtering SNPs specific for each sample collection produces consistently negative F_{IS} (excess heterozygosity) values and less of a discrepancy between averages of yearly and total values.

Area	Collection Year	Ν	MAC > 3	Н _о	H _E	F _{IS}	F _{IS} (weighted)
British Columbia	1999	49	4,198,102	0.324	0.308	-0.033	-0.050
	2004	43	3,944,777	0.349	0.323	-0.061	-0.081
	2007	50	4,227,187	0.321	0.307	-0.030	-0.048
	all years	142	7,146,268	0.220	0.210	-0.014	-0.048
Central Gulf of	1999	50	4,016,629	0.345	0.320	-0.061	-0.079
Alaska	2004	50	4,325,411	0.326	0.304	-0.049	-0.069
	2007	50	5,956,646	0.260	0.244	-0.034	-0.065
	2018	49	4,417,614	0.339	0.305	-0.087	-0.112
	all years	199	4,473,598	0.373	0.309	-0.164	-0.210
Bering Sea	2004	43	4,393,404	0.352	0.307	-0.114	-0.148
	2007	50	3,992,723	0.330	0.316	-0.022	-0.043
	all years	93	8,403,542	0.210	0.188	-0.054	-0.118
Central Aleutian	2007	37	3,588,166	0.357	0.338	-0.041	-0.055
Islands	2020	49	4,129,221	0.329	0.311	-0.039	-0.060
	all years	86	5,572,332	0.266	0.254	-0.021	-0.049
Western Aleutian	2020	50	4,065,743	0.335	0.313	-0.045	-0.069
Islands	all years	50	4,065,743	0.335	0.313	-0.045	-0.069

Table 1. Summary of diversity measures estimated from low coverage whole genome sequence data for sample collections of Pacific halibut. The table includes sample sizes (N), number of SNPs with minor allele count (MAC) \geq 3, values of mean observed heterozygosity (H_0) and mean expected heterozygosity (H_E). Mean F_{IS} (deviation of observed from expected heterozygosity) is reported as an arithmetic and weighted (by H_E) mean. These values are calculated for SNPs with a MAC \geq 3 in each sample collection.

Population structure

Principal component analysis (PCA) was carried out using PCAngsd (v1.2) (Meisner and Albrechtsen 2018) to estimate a covariance matrix from the IcWGR dataset. A MAF threshold of 0.05 was applied prior to the covariance matrix estimation. Eigendecomposition was performed in R (v4.2.2) (R Core Team 2022) using the *eigen* function. The percent variance explained for each principal component was calculated by dividing each eigenvalue associated with each principal component by the sum of all eigenvalues. To determine an appropriate number of principal components (PCs) to retain for downstream analyses, a scree plot of the first 10 eigenvalues was visually inspected and Cattell's rule (Cattell 1966) was used for this purpose. K-means clustering was performed on the retained PCs using the *kmeans* function in R. To determine the optimal number of clusters (K) present in the data, we tested a range of K values (1 to 20) and used total within-cluster sum of squares (WSS) and Bayesian information criterion (BIC) to compare the K values tested and identify the best supported number of clusters.

To compliment PCA/K-means cluster analysis, we also performed unsupervised model-based clustering and estimated individual admixture proportions using NGSadmix (v33) (Skotte et al. 2013). Similar to the PCA based clustering, we filtered SNPs with a MAF < 0.05 prior to the estimation of individual admixture proportions (cluster membership probabilities). Five replicate runs for each value of K (number of clusters, K=2-8) tested were carried out. CLUMPP (v1.1.2) (Jakobsson and Rosenberg 2007) was used to match cluster labels across the replicate runs for each value of K and obtain a mean of the permuted assignment matrices. The ΔK statistic (Evanno et al. 2005) was used to aid in identifying the best value of K. To assign an individual to a single cluster, we required that the individual's cluster membership probability be at least 0.8 to a single cluster; otherwise, that individual was categorized as unassigned.

Assignment testing

Assignment testing was also performed to assess our ability to develop a SNP panel for accurate assignment of individuals back to our baseline set of populations. We followed a simple training and holdout cross-validation procedure (see Anderson 2010; Waples 2010) by first splitting the data into a training and validation set. We randomly selected half of the individuals from each sample collection (e.g. year and geographic area) to be used for the training set and the remaining samples were set aside for validation purposes. The training set was used to select SNPs that should be informative of an individual's geographic origin and then used these samples to construct reference populations. The validation set was then used to see how the assignment tests would generalize when new samples are compared to the baseline established using the training set. First, we grouped the individuals in the training set by area and estimated pairwise F_{ST} for each SNP between all combinations of geographic areas. Pairwise F_{ST} was estimated by obtaining maximum likelihood estimates of allele and genotype frequencies for each population and calculating F_{ST} defined by Weir and Cockerham (1984) using custom software. We selected SNPs with high levels of differentiation to construct a marker panel for population assignment. Specifically, we selected the top 1,000 SNPs with the highest F_{ST} for each sample collection; however, once a SNP was included in the panel no other SNPs within 10,000 base pairs were considered to avoid selecting tightly linked SNPs containing redundant information. The lists of SNPs from each pairwise comparison were combined and subsequently any duplicate SNPs were removed. Population assignments were carried out using WGSAssign (v1.0.1) (DeSaix et al. 2024). First, a reference set of populations was established by estimating allele frequencies for each geographic area using the samples in the training set, and, second, samples in the validation set were assigned back to the reference set. Assignment accuracy was calculated as the proportion of samples in the validation set that were correctly assigned to the geographic location that they were collected from. We also conducted leave-one-out cross-validation to evaluate self-assignment rates of the fish in the training set back to their population of origin.

1.1.2. Results

Population structure

The genome-wide PCA using the filtered set of 4,793,014 SNPs (MAF \ge 0.05) revealed a lack spatial population structure (Fig. 1) among spawning groups of Pacific halibut in the Northeastern Pacific Ocean . The top two PCs capture a very small (< 1%) proportion of the total variation in the dataset (Fig. 1). By plotting individual Pacific halibut along the top two PCs, a single cluster of individuals is formed and a considerable degree of overlap of individuals among geographic areas is observed (Fig. 1), suggesting that no distinct genetic groups are apparent in the dataset. K-means clustering analysis also failed to detect discrete genetic groups. For clustering, only the first three PCs were retained following Cattell's rule (Fig. 2). Inspecting model selection measures of total within-clusters sum of squares and BIC, we see a constant and continual decay as larger K-values are tested (Fig. 3). Following the guidance of Jombart et al. (2010) on the use of BIC for selecting the best value of K, we were unable to confidently select an optimal value for K, the true number of clusters in the dataset. This is consistent with the lack of discrete genetic groups observed in Fig. 1.



Figure 1. PCA biplot of the first two PC axes for 570 Pacific halibut. Samples are colored by geographic area. Circles represent 95% confidence ellipses.



Figure 2. Scree plot of the eigenvalues for the first 10 principal components (PCs).



Figure 3. Plots of total within-clusters sum of squares (A) and Bayesian information criterion (B) for each value of K tested (1-20).

The estimation of admixture proportions and clustering implemented in NGSadmix revealed a similar lack of population structure among the sample collections used in this study since no clear groupings of samples were identified across all values of K tested (Fig. 4). Comparing values of ΔK across all values of K tested we observed the largest value associated with K=2, suggesting that the best supported number of clusters is 2 (Fig. 5). However, it is important to note that while ΔK can be a very reliable metric for identifying the true number of genetic clusters in certain scenarios, it cannot identify the best number of clusters when there is only a single cluster (i.e. when K=1) (Evanno et al. 2005) such as in the present study. The lack of population structure is also supported when examining the cluster membership coefficients of the individual samples. While the fewest number of individuals were classified as unassigned when partitioning the dataset into two clusters (K=2), it is important to note that the majority of the individuals in the dataset remained unassigned for this value of K (Fig. 6). As the value of K increased, almost all of the individuals were classified as unassigned (Fig. 6). This is most likely due to the fact that as K increases, the probabilistic assignments must be split among more categories and larger values

exceeding our threshold of 0.8 are less likely to be observed by chance. The results of the unsupervised clustering performed in NGSadmix also failed to detect discrete genetic groups of Pacific halibut in the northeast Pacific Ocean much like the PCA and K-means clustering analyses performed.



Figure 4. Barplots of individual admixture proportions estimated using NGSadmix for values of K (number of genetic clusters) ranging from 2-8. The color and height of each bar corresponds to the proportion of an individual's ancestry attributed to a specific cluster.



Figure 5. ΔK values for each value of K tested using NGSadmix.



Figure 6. Confusion matrices for cluster assignments from each value of K (2 - 8) tested using NGSadmix. We required at least 80% probability for an individual to be assigned to a single cluster, otherwise the individual was classified as unassigned or admixed.

Assignment testing

After combining the top 1,000 SNPs selected from each pairwise population comparison and removing duplicates, the resulting maker panel used for assignment testing contained 8.497 SNPs. Despite following procedures to select a subset of 8,497 SNPs to discriminate among populations (e.g. selecting the SNPs that are most differentiated among groups that we desire to discriminate amongst), we were unable to assign individuals back to their population of origin with a high degree of accuracy. Assignment success of the samples in the validation set was 34.72%. Interestingly, all of the samples in the validation-set were assigned with high confidence (> 95%) to the Central Gulf of Alaska (Fig. 7a). Evaluation of the training set using leave-one-out cross-validation yielded a 100% self-assignment rate of with all of the samples assigning back the geographic area in which they were collected (Fig. 7b). Our interpretation of the results on the complete assignment of the samples in the validation set to one particular geographic area (i.e. Central Gulf of Alaska) is that we are simply capturing noise in the training set due to the lack of genetic structure. The area with the largest number of samples is the Central Gulf of Alaska and, therefore, when split into training and validation sets, a large number of samples are available for accurate estimation of allele frequencies associated with this area. At other sampling localities, splitting the samples into two sets likely leads to less accurate allele frequency estimation for these areas due to smaller sample sizes. The allele frequencies estimated for the Central Gulf of Alaksa may be the most accurate representation of the stock as a whole, and, as a consequence of the general lack of spatial structure, individuals are being assigned to this area with a high degree of confidence



Figure 7. Confusion matrices for individual population assignments using a set of 8,497 SNPs, requiring a minimum assignment probability of 95% for an individual to be assigned to a reference population. Geographic area of origin and assigned population are respectively shown on the x and y axes. A) Count of individuals in the validation set with assignments to the reference populations established using the training set. B) Assignment counts of individuals in the training set that self-assign to the reference populations, established using leave-one-out cross-validation.

1.1.3. Conclusions

The results presented here support the notion that a single genetic group of Pacific halibut inhabits the northeast Pacific Ocean. Unsupervised clustering analyses failed to confidently identify discrete genetic groups, levels of genome-wide differentiation are low among sample collections, and genomic signatures of natural selection are shared among the sample collections included in this study, despite being collected over broad temporal and spatial scales. Furthermore, assignment testing validated with cross-validation techniques indicate limited ability to accurately assign samples back to the location in which they were sampled from. We hypothesize that the absence of distinct genetic groups among our sample collections is due to a considerable degree of geneflow among the areas sampled in this study and, consequently, to the genetically panmictic nature of the sampled Pacific halibut population.

The lack of structure observed here is not surprising given our current knowledge and biology of Pacific halibut. Annual migration rates estimated from tag recovery data suggest that there is ample opportunity for individuals to move among IPHC Regulatory Areas throughout their lives (Webster et al. 2013). Analysis of tag recovery data has shown that approximately 11% of Pacific halibut tags are recovered in a different IPHC Regulatory Area than they are released (Carpi et al. 2021). This varies by regulatory area but for most IPHC Regulatory Areas, the percentage of migrants observed exceeds 10% (Carpi et al. 2021). Additionally, strong oceanographic connectivity between the Bering Sea and Gulf of Alaska has been linked to a

considerable degree of larval exchange between these areas. It has been estimated that 47%-58% of larvae originating from spawning grounds in the Western Gulf of Alaska are transported to the Bering Sea (Sadorus et al. 2021). These rates can still be as high as 4.5%-8.6% for larvae originating from spawning grounds in the Eastern Gulf of Alaska (Sadorus et al. 2021).

The concept of a stock and the ability to define management units is central to sound management of marine fishes (Begg et al. 1999; Cadrin 2020). Advances in genomic technology have led to the development of useful and powerful tools that can aid in the delineation of management units (Bernatchez et al. 2017). Despite using very high-resolution genomic methods to characterize genomic variation in spawning groups of Pacific halibut collected over large spatial and temporal scales, the results presented here are consistent with genetic panmixia. However, while it is important to note that we cannot simply prove panmixia exists by failing to reject it, the results presented here are consistent with the current assessment practices of the Pacific halibut stock in IPHC Convention Waters which is treated as a single coastwide stock (Stewart and Hicks 2024).

2. <u>Reproduction</u>.

Research activities in this Research Area aim at providing information on key biological processes related to reproduction in Pacific halibut (maturity and fecundity) and to provide sex ratio information of Pacific halibut commercial landings. The relevance of research outcomes from these activities for stock assessment (SA) is in the scaling of Pacific halibut biomass and in the estimation of reference points and fishing intensity. These research outputs will result in a revision of current maturity schedules and will be included as inputs into the SA (<u>Appendix II</u>), and represent some of the most important biological inputs for stock assessment (please see document <u>IPHC-2021-SRB018-06</u>). The relevance of these research outcomes for the management and strategy evaluation (MSE) process is in the improvement of the simulation of spawning biomass in the Operating Model (<u>Appendix III</u>).

- 2.1. <u>Sex ratio of the commercial landings</u>. The IPHC Secretariat is finalizing the processing of genetic samples from the 2023 aged commercial landings.
- 2.2. <u>Reproductive assessment.</u> Recent sensitivity analyses have shown the importance of changes in spawning output due to changes in maturity schedules and/or skip spawning and fecundity for SA (<u>Stewart and Hicks, 2018</u>). Information on these key reproductive parameters provides direct input to the SA. For example, information on fecundity-at-age and -size could be used to replace spawning biomass with egg output as the metric of reproductive capability in the SA and management reference points. This information highlights the need for a better understanding of factors influencing reproductive biology and success of Pacific halibut. In order to fill existing knowledge gaps related to the reproductive biology of female Pacific halibut, research efforts are devoted to characterizing female reproduction in this species. Specific objectives of current studies include: 1) update of maturity schedules based on histological-based data; and 2) fecundity estimations.

2.2.1. Update of maturity schedules based on histological-based data. The IPHC Secretariat is undertaking studies to revise maturity schedules in all four IPHC Biological Regions through histological (i.e. microscopic) characterization of maturity, as reported previously. The coastwide maturity schedule (i.e. the proportion of mature females by age) that is currently used in SA was based on visual (i.e. macroscopic) maturity classification in the field (Fishery-independent Setline Survey (FISS)). To accomplish this objective, the IPHC Secretariat started collecting ovarian samples for histology during the 2022 FISS. The 2022 FISS sampling resulted in a total of 1,023 ovarian samples collected coastwide at 489 distinct FISS stations, with 440 ovarian samples from Biological Region 2, 351 samples from Biological Region 3, 181 from Biological Regions 4, and 51 samples from Biological Region 4B (Fig. 8).



Figure 8. Map of 2022 maturity samples for histology collected on FISS. Red dots indicate a distinct FISS station in which a sample was collected.

When examining the temporal component of sampling (by week), sample collection took place from the end of May (week 21) to beginning of September (week 37) 2022. Biological Region 2 had consistent collection across time (weeks 21 to 32), with Biological Region 3 having a gap in collection from weeks 28 to 31. Sample collections in Biological Regions 4 and 4B were distinctly separated due to the same FISS vessel sampling those two regions (Fig. 9).

When examining the age and length distribution of fish collected for sampling, the distribution of fish appeared to be right-skewed for both parameters, but more pronounced for age (Fig. 10). For the samples collected in 2022, the total range of ages was from 5 to 38 years old, and the total range of lengths was from 50 to 185 cm. The largest proportion of sampled fish was from 7 to 10 years old, and from 80 to 90 cm in length.



Figure 9. Timing of maturity sample collection on the 2022 FISS. The size of the bubbles indicates the number of samples collected at each bin during week of calendar year.



Figure 10. Histograms showing distribution of age and length of female Pacific halibut collected for maturity samples in the 2022 FISS.

Ovarian samples from 2022 were processed for histology and IPHC Secretariat staff finalized scoring samples for maturity using histological maturity classifications, as previously described in Fish et al. (2020, 2022). Following this maturity classification criteria, all sampled Pacific halibut females were assigned to either the mature or immature categories. Mature female Pacific halibut are deemed to have at least reached early vitellogenesis (Vtg1) for oocyte development.

Maturity ogives (i.e., the relationships between the probability of maturity determined by histological assessments and variables including IPHC Biological Region, age, fork length and net weight) were estimated by fitting generalized linear models with logit link (i.e., logistic regression). That is, if p_i is the probability that the *i*th sampled fish is mature, then the model is:

$$\log\left(\frac{p_i}{1-p_i}\right) = \beta_0 + \sum_{m=1}^M \beta_m x_{m,i}$$

where $x_{m,i}$ is the value of the *m*th variable in the model for fish *i* (e.g., age, log(age), length, etc). The β_m are the coefficients to be estimated when fitting the model.

Alternative models were compared using the Akaike information criterion (AIC, Akaike 1973), with smaller AIC values indicating better fitting models (<u>Table 2</u>). Preliminary modeling showed the models fitted with log(age) provided a better fit, with the estimated curves better matching the initial steep rise in the proportion of mature females with age, and subsequent slower increase for older fish (<u>Fig. 11a</u>). Likewise, for models that included length or weight only (i.e., not added to a model already including age), log transformation of these variables also improved model fit. Models were fitted using function glm from the stats package (R Core Team 2013) in R 4.3.2.

Significant spatial differences in maturity-at-age, -length, and -weight are apparent across the IPHC Biological Regions, with the inclusion of IPHC Biological Region in all models leading to improved fit as indicated by lower AIC values (<u>Table 2</u>). When comparing Biological Regions 2 and 3, where the majority (>77%) of samples were collected from, Biological Region 2 appears to be showing a lower proportion of mature females at any given age (Fig. 11a) and size (Figs. 12a and 13a) than Biological Region 3 that contains younger and smaller maturing females. Biological Region 4 is showing females maturating at an older age when compared to other Biological Regions (except Biological Region 2. Biological Region 4B has the steepest ogive curve for maturity-at-age, most similar to Biological Region 3, reaching asymptote of 100% mature at the youngest age, and is also showing maturing females at a larger size compared to the other three Biological Regions. These results are the first to identify spatial differences in histological Regions.

	Model	AIC
Age	Age	995.99
	Age * Region	894.24
	sqrt(Age) * Region	882.67
	log(Age) * Region	874.06
Length	Length	1038.99
	Length * Region	944.53
	log(Length) * Region	940.03
Weight	Weight	1082.99
	Weight * Region	983.65
	log(Weight) * Region	956.41

Table 2. Generalized linear model comparisons with lower Akaike information criterion (AIC) values indicating better fitting models.

The models estimated maturity curves for each IPHC Biological Region. Noting that sample size was not proportional to population size for each region, we used the estimated regional abundance proportions from IPHC's space-time modeling of FISS numbers per unit effort (NPUE) data as weights in estimating a coastwide maturity ogive (Figs. 11b, 12b, and 13b). The value of the coastwide ogive at each age, length or weight is calculated as the abundance proportion at age, length or weight times the proportion mature at age, length or weight summed across regions. For example, for age, let q_j be the estimate of the abundance proportion for Biological Region *j*, and $p_j(age)$ be the probability of maturity at age *a* estimated from fitting the model including both region and age as explanatory variables. Then the coastwide maturity probability at age is estimated by

$$p_{CW}(a) = \sum_{j=1}^{4} q_j p_j(a)$$

The modeled coastwide ogives for both maturity-at-age and -size appear to fall between the maturity ogives for Biological Regions 2 and 3. This is expected as the majority (>77%) of Pacific halibut maturity samples were collected in these two Biological Regions. Maturity is used to assign the numbers of fish at each age in the SA model to either a reproductive or non-reproductive state. The total reproductive output of these fish in the SA is then estimated by multiplying the number of reproductive fish at each age by their average somatic weight and then by the fecundity per age or body weight (currently assumed to be 1 for all body weights and ages). Therefore, defining our coastwide maturity ogive in terms of numbers of fish is consistent with its use in the SA. Conversely, defining it in terms of biomass would require converting back to maturity in numbers for use in the SA. Age, fork length and net weight at 50% maturity were calculated from the coastwide ogive using an optimizing routine in R 4.3.2 (function optim). Age at 50% maturity (A50) was calculated to be 11.3 years, similar to current estimates from macroscopic (field) data of 11.6 years. Length at 50% maturity (L50) and net weight at 50% maturity were calculated to be 91.5 cm and 6.9 kg, respectively. Current estimates of L50 using macroscopic (field) data collection is 97.6 cm (Clark and Hare 2006).

IPHC Secretariat continued to collect ovarian samples in the 2023 FISS and will do so again during the 2024 FISS. The 2023 sampling effort resulted in a total of 1,111 ovarian samples, with 403 from Biological Region 2 and 708 from Biological Region 3. Targets for 2024 are to collect 400 samples in Biological Regions 2 and 3, and 552 in Biological Region 4. These samples will allow us to investigate both spatial and temporal differences in histological-based female Pacific halibut maturity.



Figure 11. Female Pacific halibut age at maturity by IPHC Biological Region, with color shading indicating 95% CI for each IPHC Biological Region (a). In the inset (b), the coastwide ogive for age generated from estimated regional abundance proportions (thick black line) is shown without the CI to better visualize differences between the coastwide and Biological Region ogives.



Figure 12. Female Pacific halibut length at maturity by IPHC Biological Region, with color shading indicating 95% CI for each IPHC Biological Region (a). In the inset (b), the coastwide ogive for length generated from estimated regional abundance proportions (thick black line) is shown without the CI to better visualize differences between the coastwide and Biological Region ogives.



Figure 13. Female Pacific halibut net weight at maturity by IPHC Biological Region, with color shading indicating 95% CI for each IPHC Biological Region (a). In the inset (b), the coastwide ogive for net weight generated from estimated regional abundance proportions thick black line) is shown without the CI to better visualize differences between the coastwide and Biological Region ogives.





To further examine potential differences in maturity ogives among Biological Regions, we compared mature individuals using a one-way analysis of variance (ANOVA) with region as the independent variable and age, length, weight and condition factor (Fulton's K) as dependent variables (Fig. 14). Fulton's K formula was based off Froese (2006) as

$$K = (W/L^3) * 100$$

where W is the net weight in grams and L is the fork length of the fish sampled. Only mature individuals were used due to their importance in driving the observed differences in maturity ogives among Biological Regions. Age, length and weight were log() transformed to meet assumptions of normality and homogeneity. There was a statistically significant difference between Biological Regions for age (F(3, 461) =21.66, p < 0.001), length (F(3, 477) = 40.84, p < 0.001), and weight (F(3, 477) = 37.39, p < 0.001). No significant difference was found among Biological Regions for Fulton's K(F(3, 477) = 0.30, p = 0.823). A Tukey HSD post-hoc comparison test (Tukey 1949) revealed that the age of mature females sampled in Biological Regions 4 and 4B was significantly higher than that of fish sampled in Biological Regions 2 and 3 (Fig. 14). No significant differences in age were observed between Biological Regions 2 and 3, nor between Biological Regions 4 and 4B. For length and weight, mature females sampled in Biological Region 3 were significantly smaller than in all other regions, whereas mature females sampled in Biological Region 4B were significantly larger than in all other regions. With no difference in age for mature females between Biological Regions 2 and 3, the difference in modeled maturity-at-age (Fig. 11a) for these two regions is largely driven by the higher proportion of older (18+ years) immature females in Biological Region 2. The mature female size data is in direct comparison to the modeled maturity-at-length and -weight ogives (Figs. 12a and 13a), showing that Biological Region 3 has younger and smaller maturing females and Biological Region 4B has older and larger maturing females when compared to other **Biological Regions.**

Using ovarian samples collected across the summer months, we were able to compare histological ovarian development among Biological Regions to assist with the interpretation of the differences in maturity ogives across Biological Regions (Fig. 15). Females in Biological Region 2 showed a clear increase in the proportion of mature individuals from May (<20%) until August (>70%), with females advancing from Vtg1 to Vtg3 during this period (Fig. 15). In contrast, the proportion of mature females in Biological Region 3 was already high in May (>75%) and stayed elevated until September, with mature females rapidly advancing through and completing vitellogenesis by that time, as shown by the appearance of females at the GVM stage as early as July (Fig. 15). In Region 4, mature females in July appeared in a lower proportion (approx. 20%) than in Biological Regions 2 and 3 but show clear progression through all stages in vitellogenesis. In August, the proportion of mature females in Biological Region 4 increased to approximately 40%, with mature females showing increasingly more advanced vitellogenic stages reaching even its completion (Fig. 15). With only samples collected in June, mature females in Biological Region 4B appeared to undergo earlier ovarian development than females in other Biological

Regions with \sim 50% of individuals at Vtg2 or more advanced stages, showing even signs of completion of vitellogenesis. This temporal analysis of ovarian development in mature females across Biological Regions provides useful insights into the existence of obvious differences related to the timing of ovarian development in mature females throughout Convention waters. Although not all Biological Regions were similarly sampled between May and September, we observed progressively earlier advances stages in oocyte development in mature females from Biological Regions 2 to 4B. Therefore, mature females appear to develop progressively faster as they move from the easternmost area sampled (Biological Region 2) to the westernmost area sampled (Biological Region 4B). This is evident when comparing oocyte developmental stages in July across Biological Regions (except June in Biological Region 4B): Vgt1-Vtg3 in Biological Region 2, Vtg1-GVM in Biological Region 3, Vtg1 and a higher proportion of Vtg2 and Vtg3 in Biological Region 4, and a higher proportion of mature females at Vtg2, Vtg3 and GVM stages in Biological Region 4B (June). However, mature females from Biological Regions 2 and 3 are younger than those from Biological Regions 4 and 4B. Therefore, it is also conceivable that older mature females undergo reproductive development faster than younger mature females, irrespective of capture location.



Figure 15. Reproductive development of female Pacific halibut by month sampled and IPHC Biological Region. Number of samples (n) collected by month shown at the top of each figure.

With regards to maturity-at-size, mature females from Biological Region 3, because of their small size, may be allocating most of their energy to ovarian development rather than growth at a young age when compared to fish from other Biological Regions. In contrast, mature females from Biological Region 4B, given that they are older and larger, may undergo rapid ovarian development once they achieve a certain size and age. growing to a larger size at younger age and then rapidly becoming mature around the age of 10 years old. With more years of histological data over a wide geographic range, we hope to be able to compare female reproductive development differences over different space and time scales.

2.2.2. <u>Fecundity estimations.</u> The IPHC Secretariat has initiated studies that are aimed at improving our understanding of Pacific halibut fecundity. This will allow us to estimate fecundity-at-size and -age and could be used to replace spawning biomass with egg output as the metric for reproductive capability in stock assessment and management reference points. Fecundity determinations will be conducted using the auto-diametric method (Thorsen and Kjesbu 2001; Witthames et al., 2009). IPHC Secretariat staff received training on this method by experts in the field (NOAA Fisheries, Northeast Fisheries Science Center, Wood Hole, MA) in May 2023. Ovarian samples for fecundity estimations were collected during the 2023 FISS. Sampling was conducted in IPHC Biological Region 3, with a total of 456 fecundity samples collected. Using histology, as described in 2.2.1, only samples deemed mature will be processed for fecundity estimations. The IPHC Secretariat will continue to collect ovarian samples during the 2024 FISS, targeting Biological Region 2 (191 samples estimated) and Biological Region 4 (552 samples estimated) due to the reduced FISS coverage.

3. Growth.

Research activities conducted in this Research Area aim at providing information on somatic growth processes driving size-at-age in Pacific halibut. The relevance of research outcomes from these activities for stock assessment (SA) resides, first, in their ability to inform yield-per-recruit and other spatial evaluations for productivity that support mortality limit-setting, and, second, in that they may provide covariates for projecting short-term size-at-age and may help delineate between fishery and environmental effects, thereby informing appropriate management responses (Appendix II). The relevance of these research outcomes for the management and strategy evaluation (MSE) process is in the improvement of the simulation of variability and to allow for scenarios investigating climate change (Appendix III).

The IPHC Secretariat has conducted studies aimed at elucidating the drivers of somatic growth leading to the decline in SAA by investigating the physiological mechanisms that contribute to growth changes in the Pacific halibut. The two main objectives of these studies have been: 1) the identification and validation of physiological markers for somatic growth; and 2) the application of molecular growth markers for evaluating growth patterns in the Pacific halibut population.

No updates to report.

4. Mortality and Survival Assessment.

Information on all Pacific halibut removals is integrated by the IPHC Secretariat, providing annual estimates of total mortality from all sources for its stock assessment. Bycatch and wastage of Pacific halibut, as defined by the incidental catch of fish in non-target fisheries and by the mortality that occurs in the directed fishery (i.e. fish discarded for sublegal size or regulatory reasons), respectively, represent important sources of mortality that can result in significant reductions in exploitable yield in the directed fishery. Given that the incidental mortality from the commercial Pacific halibut fisheries and bycatch fisheries is included as

part of the total removals that are accounted for in stock assessment, changes in the estimates of incidental mortality will influence the output of the stock assessment and, consequently, the catch levels of the directed fishery. Research activities conducted in this Research Area aim at providing information on discard mortality rates and producing guidelines for reducing discard mortality in Pacific halibut in the longline and recreational fisheries. The relevance of research outcomes from these activities for stock assessment (SA) resides in their ability to improve trends in unobserved mortality in order to improve estimates of stock productivity and represent the most important inputs in fishery yield for stock assessment (<u>Appendix II</u>). The relevance of these research outcomes for the management and strategy evaluation (MSE) process is in fishery parametrization (<u>Appendix III</u>).

For this reason, the IPHC Secretariat is conducting two research projects to investigate the effects of capture and release on survival and to improve estimates of DMRs in the directed longline and guided recreational Pacific halibut fisheries:

- 4.1. Evaluation of the effects of hook release techniques on injury levels and association with the physiological condition of captured Pacific halibut and estimation of discard mortality using remote-sensing techniques in the directed longline fishery. After having reported on experimentally-derived estimates of discard mortality rate in the directed longline fishery (Loher et al., 2022), the second component of this study investigated the relationships among hook release techniques (e.g., gentle shake, gangion cutting, and hook stripping), injury levels, stress levels and physiological condition of released fish, as well as the environmental conditions that the fish experienced during capture. Gentle shake and gangion cutting resulted in the same injury and viability outcomes with 75% of sublegal fish in Excellent condition, while the hook stripper produced the poorest outcomes (only 9% in Excellent condition). Hook stripping also resulted in more severe injuries, particularly with respect to tearing injuries, whereas gentle shake and gangion cutting predominantly resulted in a torn cheek, effectively the injury incurred by the hooking event. Physiological stress indicators (plasma levels of glucose, lactate, and cortisol) did not significant change with viability outcomes, except for higher lactate plasma levels in fish categorized as Dead. Hematocrit was significantly lower in fish that were categorized as Dead. Furthermore, 89% of fish classified as Dead were infiltrated by sand fleas, present in several sets in deeper and colder waters. Our results indicated that avoiding the use of hook strippers and minimizing soak times in areas known to have high sand flea activity result in better survival outcomes. These results have been recently published in the peer-reviewed literature (Dykstra et al., 2024).
- 4.2. <u>Estimation of discard mortality rates in the charter recreational sector</u>. Results from a similar study conducted in fish captured using guided recreational fishery practices yielded an estimated discard mortality rate of 1.35% (95% CI 0.00-3.95%) for Pacific halibut released in Excellent viability category that were captured and released from circle hooks and tagged with acceleration-logging pop-up archival transmitting tags (sPATs). This estimate is consistent with the supposition that fish discarded in the recreational fishery from circle hooks in excellent condition have a mortality rate that is arguably lower than 3.5%, as is currently used for Excellent viability fish released in the commercial

fishery (Meyer, 2007). As this project has had a high rate of fishery recoveries to date (~11.5%, with 34 wire, 7 sPAT, 1 sPAT tether) we are investigating ways in which we can use these data to enhance the survivability modeling conducted with the sPAT data. Final data analysis and manuscript preparation are underway.

5. Fishing technology.

The IPHC Secretariat has determined that research to provide the Pacific halibut fishery with tools to reduce whale depredation is considered a high priority (<u>Appendix I</u>). This research is now contemplated as one of the research areas of high priority within the <u>5-year Program of Integrated Research and Monitoring (2022-2026</u>). Towards this goal, the IPHC secretariat is investigating gear-based approaches to catch protection as a means for minimizing whale depredation in the Pacific halibut and other longline fisheries with funding from NOAA's Bycatch Research and Engineering Program (BREP) (NOAA Awards NA21NMF4720534 and NA23NMF4720414; <u>Appendix IV</u>). The objectives of this study are 1) to work with fishermen and gear manufacturers, via direct communication and through an international workshop, to identify effective methods for protecting hook-captured flatfish from depredation; and 2) to develop and pilot test 2 simple, low-cost catch-protection designs that can be deployed effectively using current longline fishing techniques and on vessels currently operating in the Northeast Pacific Ocean.

The results and outcome of the first phase of this project were reported in the documentation provided for the SRB020 meeting: <u>IPHC-2022-SRB020-08</u>.

During the second phase of the project, the IPHC Secretariat worked with catch protection device manufacturers for the design of two different types of devices for field testing: one based on a modification of Sago Solutions SA's catch protection device (i.e., shuttle) and one based on a modification of a slinky pot (i.e., shroud) deployed on branch line gear. Pilot testing was designed to investigate (1) the logistics of setting, fishing, and hauling of the two pilot catch protection designs, and (2) the basic performance of the gear on catch rates and fish size compared to non-protected gear. Field work was conducted off Newport, OR, aboard the R/V Pacific Surveyor (56' length) in late May 2023.

5.1. <u>Characteristics of the two different catch protection devices and their performance during</u> <u>field tests</u>

5.1.1 Shuttle device. Manufactured in Norway by Sago Solutions AS, two replicate shuttle devices were modeled after the Sago Extreme model but smaller at 80% size (Fig. 16). Their dimensions are 2.60 m (8.5 ft) long by 0.80 m (2.6 ft) in diameter, each weighing approximately 100 kg (220 lb.) when empty. Typically, these devices are set with the gear; however, for this study the units were deployed from the surface, during the haulback event, by threading them onto a blank skate of gear between the control and the treatment skates.



Figure 16. Images of the prototype shuttle devices used in this study in profile (A) and frontal views (B).

Shuttle gear had a standard fixed gear skate of 100 hooks on 5.5 m (18 ft) spacing, a blank half skate (on which to thread and allow the shuttle to reach the bottom before entraining catch) followed by a second skate. Gear was allowed to soak for three hours. During the hauling of gear, the shuttle was spliced onto the blank skate of gear, after which it slid down the groundline while removing fish from the hooks, before encountering the pre-installed stopper device and returning the catch to the surface. Upon reaching the surface the shuttle was hoisted onto the vessel where it was opened, and the fish were released onto the deck (Fig. 17). All fish were released back to the sea after basic data (e.g. species, length, weight, injury) were collected.



Figure 17. Shuttle being retrieved (A), catch entrained in shuttle (B), and catch being released onto the vessel deck (C).

Small adjustments were made to protocols to attach the shuttle safely and efficiently to the groundline, and the introduction of smaller hooks and weaker gangions led to lower levels of damage to the entrained fish. Shuttles had good entrapment of catch, with sets containing the shuttle yielding similar catch rates to the control sets (Fig. 18A), as well as similar size fish of entrained catch (Fig. 18B).



Figure 18. Number of individuals (A), and fork length (B) of Pacific halibut recovered per skate of control gear or retrieved by the shuttle.

5.1.2. <u>Shroud device</u>. Shrouds were constructed in house by modifying a commercially available slinky pot by opening one end and installing a rigid cap in the other end. Shrouds were designed to slide down the branch line during haulback, clustering the snaps (and hooks) and covering any catch present (<u>Fig. 19</u>).



Figure 19. Schematic of shrouded branch line actively fishing on the seabed (A) and a constructed shroud (a modified slinky pot) (B).

Shroud treatments initially consisted of a shortened skate of groundline (180 m (591 ft)), to which six 15 m (48 ft) branch lines (each with 10 hooks snapped on 1.2 m (4 ft) spacing) were attached. Three branches included shrouds to cover the catch, and three control branches had no protective shroud. During testing this was reduced to two shroud-protected branches and two control branches, all with 0.6 m (2 ft) spacing to provide more handling time and to reduce injury risk to crew. Shrouds were deployed during the setting of the gear and were activated to slide down to cover the gear during the hauling/retrieval (Fig. 20) of the gear.



Figure 20. Shroud gear being retrieved A), skate covered by the shroud B), and a Pacific halibut and branchline hooks covered by the shroud C).

Real time adjustments in gear design and setting methods allowed for safe deployment of the branch lines and shrouds. The changes resulted in a very small effective fishing footprint of the gear on the bottom, which combined with high Pacific hagfish (*Eptatretus stoutii*) activity (reducing bait longevity/availability) resulted in minimal catch with which to establish comparisons between controls and treatments. It was concluded that several logistical issues would need to be improved to scale this up to commercial fishing and that even if logistics could be refined, the shrouds would conceivably still avail depredation opportunities to whales at the exposed end of the shroud. Therefore, continued development work on this form of catch protection is not being considered at this time.

In a third phase of this project, the IPHC Secretariat has recently received another grant from the Bycatch Reduction Engineering Program-NOAA entitled "Full scale testing of devices to minimize whale depredation in longline fisheries" (NA23NMF4720414; <u>Appendix IV</u>) to refine effective methods for protecting longline captured fish from depredation, and to complete replicates in the presence of toothed whales in known depredation hotspots to demonstrate the efficacy and safety of the gear. Field work for this project is planned during the latter part of 2024 or during the summer of 2025.

RECOMMENDATION/S

That the SRB:

a) **NOTE** paper IPHC-2024-SRB024-09 which provides a response to Recommendations and Requests from SRB023, and a report on current research activities contemplated within the IPHC's five-year Program of Integrated Research and Monitoring (2022-26).

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APPENDIX I

Integration of biological research, stock assessment (SA) and management strategy evaluation (MSE): rationale for biological research prioritization

Research areas	Research activities	Research outcomes	Relevance for stock assessment	Relevance for MSE	Specific analysis input	SA Rank	MSE Rank	Research priorization
Migration and population dynamics	Population structure	Population structure in the Convention Area	Altered structure of future stock assessments		If 4B is found to be functionally isolated, a separate assessment may be constructed for that IPHC Regulatory Area	2. Biological input	1. Biological	2
	Distribution	Assignment of individuals to source populations and assessment of distribution changes	Improve estimates of productivity	Improve parametization of the Operating Model	Will be used to define management targets for minimum spawning biomass by Biological Region	3. Biological input	validation of movement estimates and recruitment distribution	2
	Larval and juvenile connectivity studies	Improved understanding of larval and juvenile distribution	Improve estimates of productivity		Will be used to generate potential recruitment covariates and to inform minimum spawning biomass targets by Biological Region	3. Biological input	1. Biological parameterization and validation of movement estimates	2
	Histological maturity assessment	Updated maturity schedule		Improve simulation of spawning biomass in the Operating Model	Will be included in the stock assessment, replacing the current schedule last updated in 2006			1
Reproduction	Examination of potential skip spawning	Incidence of skip spawning	Scale biomass and reference point estimates		Will be used to adjust the asymptote of the maturity schedule, if/when a time- series is available this will be used as a direct input to the stock assessment	1. Biological		1
	Fecundity assessment	Fecundity-at-age and -size information			Will be used to move from spawning biomass to egg-output as the metric of reproductive capability in the stock assessment and management reference points	input		1
	Examination of accuracy of current field macroscopic maturity classification	Revised field maturity classification			Revised time-series of historical (and future) maturity for input to the stock assessment			1
Growth		Identification and application of markers for growth pattern evaluation			May inform yield-per-recruit and other spatial evaluations of productivity that support mortality limit-setting		3. Biological parameterization and validation for growth projections	5
	Evaluation of somatic growth variation as a driver for changes in size-at-age	Environmental influences on growth patterns	Scale stock Improve simulation productivity and variability and allow reference point estimates climate change	Improve simulation of variability and allow for scenarios investigating climate change	May provide covariates for projecting short-term size-at-age. May help to delineate between effects due to fishing and those due to environment, thereby informing appropriate management response			5
		Dietary influences on growth patterns and physiological condition			May provide covariates for projecting short-term size-at-age. May help to deleineate between effects due to fishing and those due to environment, thereby informing appropriate management response			5
	Discard mortality rate estimate: longline fishery	Experimentally-derived	Improve trends in unobserved mortality	Improve estimates of stock productivity	Will improve estimates of discard mortality, reducing potential bias in stock assessment results and management of mortality limits	- 1. Fishery yield		4
Mortality and survival assessment	Discard mortality rate estimate: recreational fishery	DMR			Will improve estimates of discard mortality, reducing potential bias in stock assessment results and management of mortality limits		1. Fishery parameterization	4
	Best handling and release practices	Guidelines for reducing discard mortality			May reduce discard mortality, thereby increasing available yield for directed fisheries	2. Fishery yield		4
Fishing technology	Whale depredation accounting and tools for avoidance	New tools for fishery avoidance/deterence; improved estimation of depredation mortality	Improve mortality accounting	Improve estimates of stock productivity	May reduce depredation mortality, thereby increasing available yield for directed fisheries. May also be included as another explicit source of mortality in the stock assessment and mortality limit setting process depending on the estimated magnitude	1. Assessment data collection and processing		3



<u>APPENDIX II</u>

List of ranked biological uncertainties and parameters for stock assessment (SA) and their links to biological research areas and research activities

SA Rank	Research outcomes	Relevance for stock assessment	Specific analysis input	Research Area	Research activities
1. Biological input	Updated maturity schedule		Will be included in the stock assessment, replacing the current schedule last updated in 2006		Histological maturity assessment
	Incidence of skip spawning	Scale biomass and	Will be used to adjust the asymptote of the maturity schedule, if/when a time-series is available this will be used as a direct input to the stock assessment		Examination of potential skip spawning
	Fecundity-at-age and -size information	reference point estimates	Will be used to move from spawning biomass to egg-output as the metric of reproductive capability in the stock assessment and management reference points	Reproduction	Fecundity assessment
	Revised field maturity classification		Revised time-series of historical (and future) maturity for input to the stock assessment		Examination of accuracy of current field macroscopic maturity classification
2. Biological input	Stock structure of IPHC Regulatory Area 4B relative to the rest of the Convention Area	Altered structure of future stock assessments	If 4B is found to be functionally isolated, a separate assessment may be constructed for that IPHC Regulatory Area	Genetics and	Population structure
3. Biological input	Assignment of individuals to source populations and assessment of distribution changes	Improve estimates	Will be used to define management targets for minimum spawning biomass by Biological Region	Genomics	Distribution
	Improved understanding of larval and juvenile distribution		Will be used to generate potential recruitment covariates and to inform minimum spawning biomass targets by Biological Region	Migration	Larval and juvenile connectivity studies
1. Assessment	Sex ratio-at-age	Scale biomass and	Annual sex-ratio at age for the commercial fishery fit by the stock assessment	Denneduction	Sex ratio of current commercial landings
and processing	Historical sex ratio-at-age	fishing intensity	Annual sex-ratio at age for the commercial fishery fit by the stock assessment	Reproduction	Historical sex ratios based on archived otolith DNA analyses
2. Assessment data collection and processing	New tools for fishery avoidance/deterence; improved estimation of depredation mortality	Improve mortality accounting	May reduce depredation mortality, thereby increasing available yield for directed fisheries. May also be included as another explicit source of mortality in the stock assessment and mortality limit setting process depending on the estimated magnitude	Mortality and survival assessment	Whale depredation accounting and tools for avoidance
1. Fishery yield	Physiological and behavioral responses to fishing gear	Reduce incidental mortality	May increase yield available to directed fisheries	Mortality and survival assessment	Biological interactions with fishing gear
2. Fishery yield	Guidelines for reducing discard mortality	Improve estimates of unobserved mortality	May reduce discard mortality, thereby increasing available yield for directed fisheries	Mortality and survival assessment	Best handling practices: recreational fishery

<u>APPENDIX III</u>

List of ranked biological uncertainties and parameters for management strategy evaluation (MSE) and their links to biological research areas and research activities

MSE Rank	Research outcomes	Relevance for MSE	Research Area	Research activities	
1. Biological parameterization and validation of movement estimates	Improved understanding of larval and juvenile distribution	Improve parametization of the	Migration	Larval and juvenile connectivity studies	
	Stock structure of IPHC Regulatory Area 4B relative to the rest of the Convention Area	Operating Model		Population structure	
2. Biological parameterization and	Assignment of individuals to source populations and assessment of distribution changes	Improve simulation of recruitment variability and parametization of recruitment distribution in the Operating Model	Genetics and Genomics	Distribution	
validation of recruitment variability and distribution	Establishment of temporal and spatial maturity and spawning patterns	Improve simulation of recruitment variability and parametization of recruitment distribution in the Operating Model	Reproduction	Recruitment strength and variability	
3. Biological parameterization and validation for growth projections	Identification and application of markers for growth pattern evaluation		Growth		
	Environmental influences on growth patterns	Improve simulation of variability and allow for scenarios investigating climate change		Evaluation of somatic growth variation as a driver for changes in size-at-age	
	Dietary influences on growth patterns and physiological condition				
1. Fishery parameterization	Experimentally-derived DMRs	Improve estimates of stock productivity	Mortality and survival assessment	Discard mortality rate estimate: recreational fishery	



INTERNATIONAL PACIFIC HALIBUT COMMISSION

IPHC-2024-SRB024-09

APPENDIX IV

Summary of current external research grants

Project #	Grant agency	Project name	PI	Partners	IPHC Budget (\$US)	Management implications	Grant period
1	Bycatch Reduction Engineering Program - NOAA	Full scale testing of devices to minimize whale depredation in longline fisheries (NA23NMF4720414)	IPHC	NOAA Fisheries - Alaska Fisheries Science Center (Seattle)	\$199,870	Mortality estimations due to whale depredation	November 2023 – April 2025
2	Alaska Sea Grant (pending award)	Development of a non-lethal genetic-based method for aging Pacific halibut (R/2024-05)	IPHC, Alaska Pacific Univ. (APU)	Alaska Fisheries Science Center-NOAA (Juneau)	\$60,374	Stock structure	February 2024- January 2026
Total awarded (\$)							